

A Report on Whole Sediment Toxicity of Sediments from Sites on the Ottawa River
to *Hyalella azteca* and *Lumbriculus variegatus* for the Great Lakes National Program Office

Bioassay Numbers

98-2133-CO:	Lab Control	98-2130-NW:	Site 13/Ottawa River, Upst. R.R. Trestle
98-2134-NW:	Site 09/Ottawa River, Dwst. Summit St.	98-2131-NW:	Site 14/Ottawa River @ Lagrange St.
98-2123-NW:	Lab Control	98-2132-NW:	Site 15/Ottawa River @ Berdan Ave.
98-2124-NW:	Site 10/Ottawa River @ I-75	98-2143-CO:	Lab Control
98-2125-NW:	Site 11/Ottawa River Dwst. Stickney Ave.	98-2144-NW:	Site 16/Ottawa River/@ Jeep Parkway
98-2126-NW:	Site 12/Ottawa River Adj. to Stickney Ave.	98-2145-NW:	Site 17/Ottawa River/@ Auburn Ave.
98-2129-CO:	Lab Control	98-2146-NW:	Site 18/Ottawa River/@ University of Toledo

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INTRODUCTION

Sediment samples were collected from ten sites on the Ottawa River to assess sediment toxicity and to determine whether future bioaccumulation testing is possible for use in a Great Lakes National Project Office (GLNPO) Maumee Basin survey. Sediments were collected from the Ottawa River on five dates in the month of August. Ottawa River sediment sample identification and collection dates are in Table 1. The sediments were collected from each location using a Petite Ponar or Ekman Dredge at a depth of approximately 10 centimeters (cm) where possible. Sampling locations and other pertinent information are recorded on the sediment sample submission forms (Appendix C). The aliquots of sediment from each subsite were thoroughly stirred to achieve a homogenous mixture before portions were transferred to 1000 milliliter (ml) opaque white polyethylene containers. Insulated coolers containing water ice were used to transport samples to the laboratory at approximately 4°C. Upon receipt at the laboratory, samples were placed in dark storage at 4°C until testing. *Hyalella azteca* was used as the test organism for the 10-day sediment toxicity tests. *Lumbriculus variegatus* was used as the test organism for the 96-hour sediment toxicity/bioaccumulation screening tests. Four sets of *L. variegatus* bioassays were conducted based upon the date of sediment receipt. Sediment from the Ottawa River University of Toledo site was used as the reference site and primary control. One *H. azteca* bioassay was conducted with the downstream of Summit Street sediment on 17-28 August 1998. A laboratory control sediment comprised of a 3:1 ratio sand:kaolin fine clay was used as the primary control during the *H. azteca* bioassay.

General Procedures

Test containers for both organisms were 300 ml glass beakers that were 115 millimeters (mm) in height and 75 mm in diameter. Each replicate contained 100 ml of sediment and 175 ml of overlying water. An approximately 20 mm long groove was cut into the top of each beaker and covered with stainless steel screen. This was to allow old overlying water to drain, while preventing test organisms from escaping during test solution renewal. Sediment depth was approximately 35 mm. Overlying water was approximately 65 mm in depth. Rearing unit water (carbon-filtered Columbus, Ohio tapwater) was used as overlying water.

Sediment into test chambers: Sediment sample containers were removed from the refrigerator approximately 24-hours prior to animal addition (day minus one) and warmed to room temperature. A stainless steel spoon was used to transfer the sediment to a stainless steel beaker for homogenization. Sediments were thoroughly homogenized by hand using the stainless steel spoon. Sediment was added via 60 cc plastic syringes and/or spoons, depending on the composition of the sediment, to randomly selected test containers. A separate clean spoon, beaker, and syringe was used for each site. The 175 ml of overlying water was gently poured down the inner wall of the test vessel to minimize disturbing the sediment. Test containers were covered with double-strength glass or plexiglass and placed in an incubator at 25° 2EC (*H. azteca*) and at 23° 2EC (*L. variegatus*) overnight. The incubator photoperiod was 16 hours light and 8 hours dark. Illumination was between 245-1670 lux for the bioaccumulation screen test.

Animal addition (day zero): Ten adult *H. azteca* were randomly added to each of eight replicate test containers per site and control. Initial average dry weight of these organisms was 0.034-0.009 milligrams (mg). Ten adult *L. variegatus* were randomly added to each of four replicate test containers per site and control. Initial average dry weight of these organisms ranged from 0.773-0.905 mg.

Feeding: Food for the *H. azteca* toxicity test was a 1:1 ratio of Ceraphyll (at 5 grams/liter [g/L] concentration) and *Selenastrum capricornutum* algae. Algal cell density was 1.0×10^8 cells/ml. Each replicate received 1.5 ml of test food per day delivered after the morning water renewal. *L. variegatus* were not fed during the test.

Renewal of overlying water: Overlying water was renewed twice daily at approximately 12-hour intervals (0530 hours and 1730 hours). At the time of renewal, water quality measurements (temperature,

dissolved oxygen [DO], pH, and conductivity) were taken on the “new” and “old” water, and observations were recorded. A 60 cc syringe was used to remove 30 ml of overlying water from each replicate in a group and composited for physicochemical measurements. During the *H. azteca* test, hardness and alkalinity of the new overlying water was measured on days 0, 4, and 7. Ammonia concentrations in the old overlying water was measured from each treatment on days 2 and 9 for the *H. azteca* tests. Hardness, alkalinity, and ammonia of the old overlying water was measured from each treatment in the *L. variegatus* tests on days 0 and 4. Modified splitter chambers, as described by Zumwalt et al. (1994), were used for renewing overlying water.

Observations were made for mortality and avoidance of sediment. Appearance of any indigenous organisms was recorded and these were removed upon discovery.

Test end: Aliquots of overlying water were removed for water quality measurements. Organisms evident above the sediment surface were removed using a pipette. In order to count surviving animals, sediments were run through number 50 or 60 μm sieves. Water remaining in the beaker was swirled to suspend the top 5-10 mm of sediment. The slurry was poured through the sieve. Water was added to the remaining sediment, and the suspension process was repeated until all sediment was removed from each replicate. Contents of each sieve was back-flushed into a glass crystallizing dish and examined on a light table for surviving organisms. Number of live, dead, and missing organisms for each replicate was recorded. Missing organisms were presumed to have died and decayed beyond recognition. Immotile organisms isolated from the sediment surface or from sieved material were considered dead.

Acceptability of test results: Results of a test were considered acceptable if mean survival in the laboratory control sediment was 80 percent or greater. Container temperature must be 25° C for *H. azteca* and 23° C for *L. variegatus* at all times during the test. The mean dissolved oxygen concentration should be greater than 40 percent of saturation and no single measurement should drop below 40 percent saturation. Hardness, alkalinity, pH, and ammonia should not vary by more than 50 percent during the test.

Analysis: The null hypothesis in analyzing data from the tests was that there was no significant difference between the sediments of concern and the reference sediment. The alternative hypothesis is that the sediment(s) of concern are toxic relative to the reference sediment. Data were tested for normality using the Shapiro-Wilk's test followed by Bartlett's test and Hartley's test for homogeneity of variance, if normally distributed. Data with homogenous variances were then subjected to analysis of variance followed by Fisher's LSD where a comparison-wise alpha was used. Data not normally distributed or with heterogenous variance were transformed using either the arc sine square root (Y) or natural logarithms. Steel's many-one rank test was employed if transformations did not result in passing Shapiro-Wilk's test of normality. The decision tree used for analysis of the survival and growth, as measured by dry weight, data is presented in Figure 1. The alpha levels used for tests of assumptions are in Table A.1. Toxstat was used for most statistical tests. An in-house program was written and used for Fisher's LSD.

RESULTS AND DISCUSSION

The Ottawa River downstream of Summit Street sediment was not acutely toxic to *H. azteca*. Survival of *H. azteca* in the Ottawa River downstream of Summit Street site was 96.3 percent. Survival in the laboratory control was 95 percent. Since the Summit Street site was the only site on the Ottawa River in which *H. azteca* sediment bioassays were conducted, comparisons of survival and growth were done versus the laboratory control instead of a field reference. Formal statistical comparisons were forgone because survival and growth were greater in the Summit Street sediment versus the laboratory control. The high rate of survival and an approximately six fold increase in amphipod weight in the Summit Street sediment indicate the sediment is not acutely toxic to *H. azteca*. Dissolved oxygen of water overlying the Ottawa River site in the *H. azteca* test ranged from 4.5-7.0 mg/L and remained above 40 percent of saturation. Ammonia concentration in the overlying water was reduced by more than 50 percent between

days 2 and 9. Other physicochemical parameters are listed in the summary form and on the attached bench sheets.

The Ottawa River sediments were not acutely toxic to *L. variegatus*. After four days of exposure, *L. variegatus* survival was 100 percent in all of the Ottawa River sediments except those collected downstream of Stickney Avenue and at Auburn Avenue. Survival for these two sites was 97.5 and 92.5 percent, respectively. Survival of *L. variegatus* in the laboratory control sediment ranged from 95 to 100 percent. Comparison of each of the nine Ottawa River sites to the University of Toledo reference station using Steel's many one-rank test did not indicate a significant difference at an alpha level of 0.05. Dissolved oxygen of water overlying the Ottawa River sediments ranged from 5.2-7.2 mg/L and remained above 40 percent of saturation. Ammonia level in the overlying water was reduced in all treatments from days 0 and 4. Other physicochemical parameters are listed in the summary form and on the attached bench sheets.

Results of these bioassays indicate the Ottawa River sediments were not toxic to either test organism. Because the sediments were not acutely toxic to *L. variegatus* it is possible to conduct future bioaccumulation tests with this species.

Table 1. Ottawa River site identification and collection dates

Sample Site	Bioassay Number	Date Collected	Dates Tested
Lab Control	98-2133-CO		17-28 August 1998
Site 09/Downstream Summit Street	98-2134-NW	12 August 1998	
Lab Control	98-2123-CO	3 August 1998	8-12 August 1998
Site 10/@ I-75	98-2124-NW	3 August 1998	
Site 11/Downstream of Stickney Avenue	98-2125-NW	3 August 1998	
Site 12/Adjacent to Stickney Avenue	98-2126-NW	3 August 1998	
Lab Control	98-2129-CO		12-16 August 1998
Site 13/Upstream of Railroad Trestle	98-2130-NW	10 August 1998	
Site 14/@ Lagrange Street	98-2131-NW	10 August 1998	
Site 15/@ Berdan Avenue	98-2132-NW	10 August 1998	
Lab Control	98-2143-CO		24-28 August 1998
Site 16/@ Jeep Parkway	98-2144-NW	18 August 1998	
Site 17/@ Auburn Avenue	98-2145-NW	18 August 1998	
Site 18/@ University of Toledo (reference station)	98-2146-NW	19 August 1998	

Table 2. Ottawa River sediment bioassay summary information

Sample Site	Physical Description [†]	Growth (mg/L)	Mean Survival (%)	
		<i>H. azteca</i>	<i>H. azteca</i>	<i>L. variegatus</i>
Laboratory Control	3:1 Sand:Kaolin Fine Clay	0.14	95.0	100
Site 09/Downstream Summit Street	Brown fine silt	0.22	96.3	100

Laboratory Control	3:1 Sand:Kaolin Fine Clay	-	-	100
Site 10/@ I-75	Fine silt sands, dark grey, clay	-	-	100
Site 11/Downstream of Stickney Ave.	Silty sand, grey, oily sheen	-	-	97.5
Site 12/Adjacent to Stickney Avenue	Silty sands, grey color	-	-	100

Laboratory Control	3:1 Sand:Kaolin Fine Clay	-	-	100
Site 13/Upstream of Railroad Trestle	Silty clay dark brown/grey	-	-	100
Site 14/@ Lagrange Street	Grey silty sand with sheen	-	-	100
Site 15/@ Berdan Avenue	Grey sandy silts	-	-	100

Laboratory Control	3:1 Sand:Kaolin Fine Clay	-	-	95.0
Site 16/@ Jeep Parkway	Silty fine sand and coarse pebbles	-	-	100
Site 17/@ Auburn Avenue	Fine silty sand, coarse sand	-	-	92.5
Site 18/@ University of Toledo (reference station)	Course sand with silts, compacted fine silts, soft silts with sand	-	-	100

[†] All descriptions, except the laboratory controls, are from the sample submission forms.

Table 3. Analysis of Ottawa River *L. variegatus* survival data excluding the laboratory control sediment using Steel's Many-One Rank Test

STEELS MANY - ONE RANK TEST Ho:Control<Treatment						
Group	Identification	Mean	Rank Sum	Critical Value	df	Sig
1	Univ. of Toledo/ref.	1.000				
2	Dwst Summit St.	1.000	18.00	None	4	
3	@ I-75	1.000	18.00	None	4	
4	Dwst. Stickney Ave.	0.975	16.00	None	4	
5	Adj. to Stickney Ave.	1.000	18.00	None	4	
6	Upst. R.R. Trestle	1.000	18.00	None	4	
7	@ Lagrange St	1.000	18.00	None	4	
8	@ Berdan Ave.	1.000	18.00	None	4	
9	@ Jeep Parkway	1.000	18.00	None	4	
10	@ Auburn Ave.	0.925	14.00	None	4	

Critical values use k = 9, are 1 tailed, and alpha = 0.05.
Data were not normally distributed.

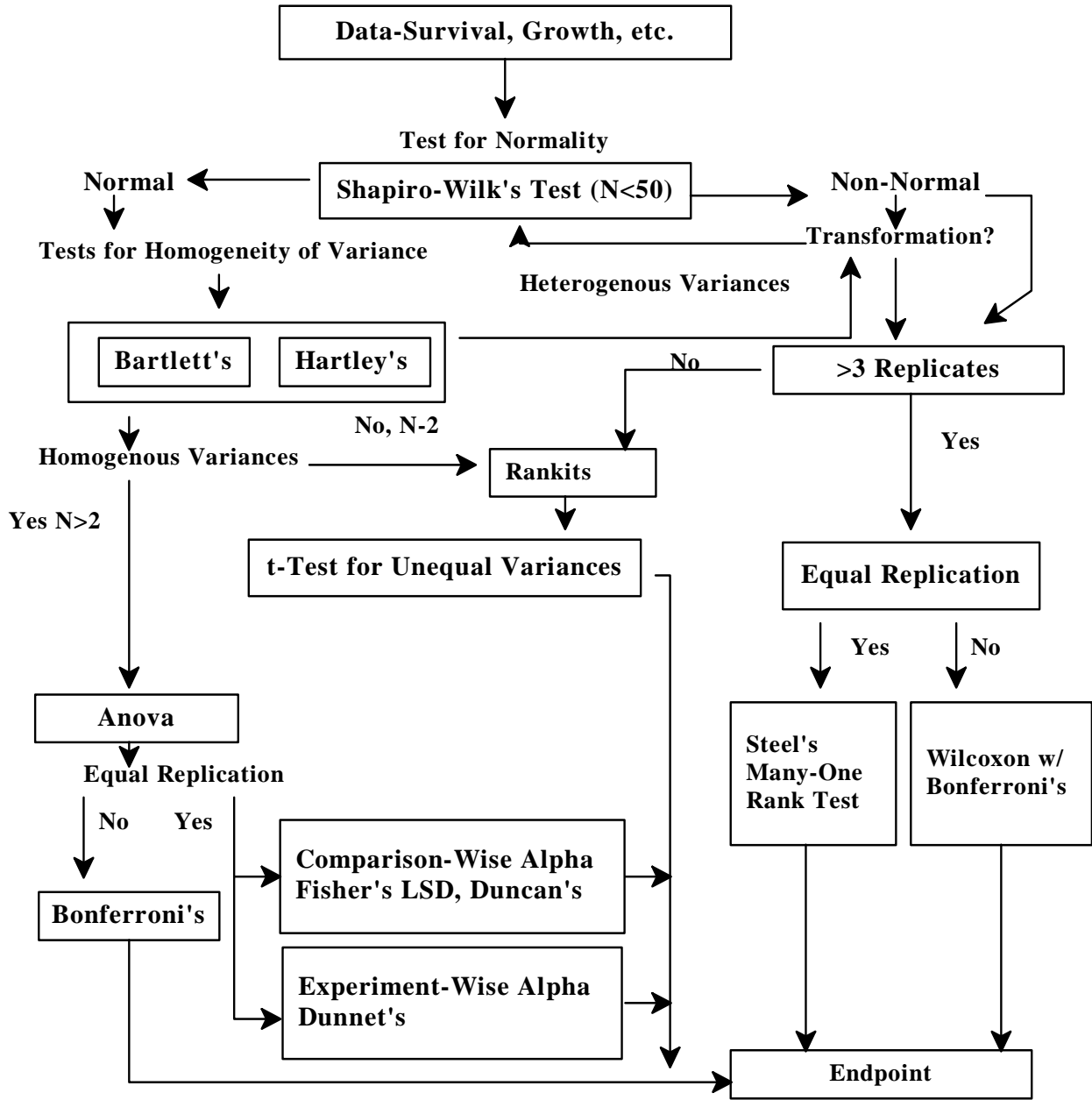


Figure 1. Decision tree for analysis of survival and growth data subjected to hypothesis testing.

**OHIO ENVIRONMENTAL PROTECTION AGENCY
Whole Sediment Toxicity Bioassay Report Form**

Report Date: 3 September 1998

Bioassay Report Number(s): 98-2133-CO < 98-2134-NW

Investigators: David L. Youngerman and Jonathan C. McLaughlin

Site Number and identification:

Site 09/Ottawa River downstream of Summit Street

Collected by: Brent Kuenzli, Katie McKibben, and Wendy Ordway, DSW, NWDO, Ohio EPA

Bioassay type:	Whole-sediment toxicity test with renewal of overlying water
Duration of test:	10-days
Beginning date:	17 August 1998, 0630 hours

Test organisms:	<i>Hyalella azteca</i>
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<i>Hyalella azteca</i> Data:	n = 160. Number of <i>H. azteca</i> used in estimating initial dry weights.		
	Mean	Standard Deviation	Range
Dry Weight (milligrams):	0.034	0.009	0.022-0.040
Age of Organisms: Born 5-7 August 1998, 10-12 days old at test initiation			

Individual test organisms are randomly placed in the test containers. Ten *H. azteca* are in a test container.

Test Containers and Sediment:						
Test Containers			Sediment		Overlying Water	
Number/Group	Volume (ml)	Dimensions base*height (mm)	Volume (ml)	Depth (mm)	Volume (ml)	Depth (mm)
4	300	75*115	100	35	175	65

Overlying water:	Rearing unit water (carbon-filtered, aged, Columbus, Ohio tapwater)
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Laboratory control:	3:1 by weight - Sand to Kaolin fine clay
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One set of laboratory controls is routinely used when more than one sample of sediment is tested.

All test containers are chemically-clean glass beakers previously soaked or rinsed in deionized water.

A 20 mm slot is cut into the side of the beakers and covered with stainless steel screen (425 F m mesh), allowing overlying water to drain while preventing the escape of test organisms.

Photoperiod :	Automatically maintained at 16 hours light - 8 hours dark
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Test Temperature:	Maintained at 25" 2EC
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Samples taken for chemical analyses at time of bioassay sample collection:	Yes, results attached.
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Chemical analyses performed by:	Ohio EPA Division of Environmental Services.
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Adverse effects measured in the test are death, lethargy, and avoidance of sediment. Death is the cessation of all visible movement with no response to gentle prodding. A lethargic organism is sluggish in movement. Sediment avoidance is when *H. azteca* do not burrow below the sediment surface. A sediment is considered toxic if the number of *H. azteca* in the sediment is significantly reduced relative to the control(s). Also, organisms should burrow into the sediment. Test results are invalid if more than 20 percent of the test organisms in the control exhibit adverse effects.

Survival Results: *Hyalella azteca* whole sediment toxicity tests of Ottawa River sediments conducted on 17-28 August 1998

Replicate	98-2133-CO Laboratory Ctrl	98-2134-NW Downstream Summit St.
A	10	8
B	9	10
C	9	10
D	9	9
E	10	10
F	10	10
G	9	10
H	10	10
Mean	9.50	9.63
Standard Dev.	0.535	0.744

Growth Results: *Hyalella azteca* whole sediment toxicity tests of Ottawa River sediments conducted on 17-28 August 1998

Replicate	98-2133-CO Laboratory Ctrl	98-2134-NW Downstream Summit St.
A	0.135	0.285
B	0.141	0.199
C	0.126	0.222
D	0.160	0.212
E	0.140	0.223
F	0.139	0.222
G	0.139	0.181
H	0.114	0.206
Mean	0.14	0.22
Standard Dev.	0.013	0.030

Relevant information: Since the Summit Street site was the only site on the Ottawa River in which *H. azteca* sediment bioassays were conducted, comparisons of survival and growth were done versus the laboratory control instead of a field reference. Formal statistical comparisons were forgone because survival and growth were greater in the Summit Street sediment versus the laboratory control. The high

rate of survival and an approximately six fold increase in amphipod weight in the Summit Street sediment indicate the sediment is not acutely toxic to *H. azteca*. Physicochemical parameters recorded during the test are on summary tables on the next page and attached bench sheets.

Results: Physicochemical parameters of overlying water during *Hyalella azteca* whole sediment toxicity tests of Ottawa River sediments conducted on 17-28 August 1998

Hardness and alkalinity of new overlying water

Test Day	Hardness as mg/L CaCO ₃	Alkalinity as mg/L CaCO ₃
0	124	37
4	112	47
7	116	43
Mean ± Standard Deviation	117 " 6.1	42 " 5.0

Alkalinity, hardness, and ammonia concentrations in the old overlying water:

Test Site	Test Day	Ammonia (mg/L)
Laboratory Control	2	0.96
	9	0.32
Site 09 Downstream Summit Street	2	0.61
	9	0.22

Ranges of physicochemical parameters in the old overlying water:

Test Site	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (SU)	Conductivity (µmhos/cm)
Laboratory Control	22.6*/24.0-25.9	6.1-8.3	7.39-7.97	475-524
Site 09/Dwst. Summit St.	23.0*/24.0-26.2	4.5-7.0	7.32-7.85	490-537

*Sediments accidentally placed in the wrong incubator, set at 23° 2EC, overnight from day 2-3.

**OHIO ENVIRONMENTAL PROTECTION AGENCY
Whole Sediment Toxicity Bioassay Report Form**

Report Date: 3 September 1998

Investigators: David L. Youngerman and Jonathan C. McLaughlin

Site Number and identification:	Ottawa River	Bioassay Number:
Site 09/Downstream Summit Street		98-2134-NW
Site 10/@ I-75		98-2124-NW
Site 11/Downstream Sitckney Avenue		98-2125-NW
Site 12/Adjacent to Stickney Avenue		98-2126-NW
Site 13/Upstream Railroad Trestle		98-2130-NW
Site 14/@ Lagrange Street		98-2131-NW
Site 15/@ Berdan Avenue		98-2132-NW
Site 16/@ Jeep Parkway		98-2144-NW
Site 17/@ Auburn Avenue		98-2145-NW
Site 18/@ University of Toledo (reference station)		98-2146-NW

Collected by: Brent Kuenzli, Katie McKibben, and Wendy Ordway DSW, NWDO, Ohio EPA

Bioassay type:	Whole-sediment toxicity test with renewal of overlying water
Duration of test:	96-hours
Beginning date(s):	8 August 1998, 0635 hours; 12 August 1998, 0605 hours; 17 August 1998, 0655 hours; and 24 August 1998, 0620 hours

Test organisms:	<i>Lumbriculus variegatus</i> originally provided by Aquatic Research Organisms® on 23 June 1998 and maintained in-house in the Ohio EPA Bioassay Section rearing unit.
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<i>Lumbriculus variegatus</i> Data:	n = 40. Number of <i>L. variegatus</i> used in estimating initial dry weights.		
	Mean	Standard Deviation	Range
Dry Weight (milligrams):	0.86	0.086	0.74-1.05
Age of Organisms: Adults; cultures initiated on 23 June 1998			

Individual test organisms are randomly placed in the test containers. Ten *L. variegatus* are in a test container.

Test Containers and Sediment:						
Test Containers			Sediment		Overlying Water	
Number/Group	Volume (ml)	Dimensions base*height (mm)	Volume (ml)	Depth (mm)	Volume (ml)	Depth (mm)
4	300	75*115	100	35	175	65

Overlying water:	Rearing unit water (Carbon-filtered, aged, Columbus, Ohio tapwater)
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Laboratory control:	3:1 by weight - Sand to Kaolin fine clay
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One set of laboratory controls is routinely used when more than one sample of sediment is tested.

All test containers are chemically-clean glass beakers previously soaked or rinsed in deionized water.

A 20 mm slot is cut into the side of the beakers and covered with stainless steel screen (425 F m mesh), allowing overlying water to drain while preventing the escape of test organisms.

Photoperiod :	Automatically maintained at 16 hours light - 8 hours dark
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Test Temperature:	Maintained at 23° 2EC
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Samples taken for chemical analyses at time of bioassay sample collection:	Yes, results attached.
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Chemical analyses performed by:	Ohio EPA Division of Environmental Services.
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Adverse effects measured in the test are death, lethargy, and avoidance of sediment. Death is the cessation of all visible movement with no response to gentle prodding. A lethargic organism is sluggish in movement. Sediment avoidance is when *L. variegatus* do not burrow below the sediment surface. A sediment is considered toxic if the number of *L. variegatus* in the sediment is significantly reduced relative to the control(s). Also, organisms should burrow into the sediment. Avoidance of sediment by the organisms may decrease bioaccumulation. Test results are invalid if more than 20 percent of the test organisms in the control exhibit the adverse effects.

Results: *Lumbriculus variegatus* whole sediment toxicity tests of Ottawa River sediments conducted August 1998

Bioassay Number Treatment	Number of Survivors Recovered				Mean (3 (a-d)/4)	Standard Deviation
	Replicate					
	a	b	c	d		
98-2133-CO Laboratory Control	10	10	10	10	10	0
98-2134-NW Site 09/Downstream Summit Street	10	10	10	10	10	0

98-2123-CO Laboratory Control	10	10	10	10	10	0
98-2124-NW Site 10/@ I-75	10	10	10	10	10	0
98-2125-NW Site 11/Downstream Stickney Avenue	9	10	10	10	9.75	0.5
98-2126-NW Site 12/Adjacent to Stickney Avenue	10	10	10	10	10	0

98-2129-CO Laboratory Control	10	10	10	10	10	0
98-2130-NW Site 13/Upstream Railroad Trestle	10	10	10	10	10	0
98-2131-NW Site 14/@ Laorange Street	10	10	10	10	10	0
98-2132-NW Site 15/@ Berdan Avenue	10	10	10	10	10	0

98-2143-CO Laboratory Control	10	9	10	9	9.5	0.577
98-2144-NW Site 16/@ Jeep Parkway	10	10	10	10	10	0
98-2145-NW Site 17/@ Auburn Avenue	9	10	10	8	9.25	0.957
98-2146-NW Site 18/@ Univ. of Tol. (reference station)	10	10	10	10	10	0

Relevant information: Comparison of the Nine Ottawa River sites to the University of Toledo reference station sediment using Steel's many one-rank test indicated no significant difference at an alpha level of 0.05. Physicochemical parameters recorded during the test are on summary tables on the next page and on attached bench sheets.

Results (cont.): Physicochemical parameters of overlying water during *Lumbriculus variegatus* sediment toxicity tests of Ottawa River sediments

Alkalinity, hardness, and ammonia concentrations in the old overlying water:

Test Site	Test Day	Alkalinity (mg/L CaCO ₃)	Hardness (mg/L CaCO ₃)	Ammonia (mg/L)
Laboratory Control (17-28 August)	0	37	124	0.13
	4	35	120	0.02
Site 09/Dwst. Summit St.	0	60	136	1.40
	4	52	132	0.18
Laboratory Control (9-12 August)	0	36	120	0.12
	4	34	112	0.06
Site 10/@ I-75	0	56	132	2.93
	4	58	132	1.07
Site 11/Dwst. Stickney Ave.	0	58	132	1.11
	4	60	136	0.95
Site 12/Adj. Stickney Ave.	0	62	136	3.05
	4	64	160	2.07
Laboratory Control (12-16 August)	0	32	120	0.16
	4	39	120	0.07
Site 13/Upst. R.R. Trestle	0	69	140	3.17
	4	56	136	0.92
Site 14/@ Lagrange Street	0	76	144	3.29
	4	61	128	1.65
Site 15/@ Berdan Ave.	0	67	136	0.92
	4	59	136	0.73
Laboratory Control (24-28 August)	0	32	116	0.15
	4	40	116	0.00
Site 16/@ Jeep Parkway	0	53	144	1.16
	4	59	140	0.44
Site 17/@ Auburn Ave.	0	64	132	0.50
	4	57	140	0.00
Site 18/@ Univ. of Toledo	0	59	140	0.15
	4	59	132	0.06

Results (cont.): Physicochemical parameters of overlying water during *Lumbriculus variegatus* sediment toxicity tests of Ottawa River sediments

Ranges of physicochemical parameters in the old overlying water:

Test Site	Temperature (EC)	Dissolved Oxygen (mg/L)	pH (SU)	Conductivity (F mhos/cm)
Laboratory Control (17-28 August 1998)	22.0-23.3	7.7-8.1	7.47-8.19	484-529
Site 09/Dwst. Summit St.	22.0-23.3	6.1-6.9	7.36-7.78	501-549

Laboratory Control (8-12 August 1998)	22.3-22.9	7.7-8.1	7.30-7.84	510-517
Site 10/@ I-75	21.9-22.8	6.5-7.0	7.26-7.74	524-539
Site 11/Dwst. Stickney Ave.	21.9-22.9	6.1-7.0	7.28-7.64	523-542
Site 12/Adj. to Stickney Ave.	22.0-22.9	5.7-6.9	7.28-7.69	528-561

Laboratory Control (12-16 August 1998)	22.0-23.3	7.6-8.1	7.47-7.90	507-524
Site 13/Upst. R.R. Trestle	21.9-22.9	6.0-7.1	7.31-7.73	532-569
Site 14/@ Lagrange St.	21.9-22.7	5.3-6.5	7.30-7.70	527-579
Site 15/@ Berdan Ave.	22.0-23.1	5.6-6.8	7.31-7.69	520-547

Laboratory Control (24-28 August 1998)	22.9-24.2	7.1-7.9	7.51-8.04	479-496
Site 16/@ Jeep Parkway	22.7-23.9	5.3-6.8	7.36-7.95	498-546
Site 17/@ Auburn Ave.	22.6-23.9	5.2-6.5	7.37-7.91	502-533
Site 18/@ University of Toledo (reference station)	22.8-23.9	6.1-7.2	7.38-7.91	496-533

References

US EPA 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024. June 1994.

Zumwalt, D.C., Dwyer, F.J., Greer, I.E., and Ingersoll, C.G. A water-renewal system that accurately delivers small volumes of water to exposure chamber. *Environ. Toxicol. Chem.*: 1311-1314, 1994.

**Appendix A.
Statistical Analysis**

Table A. 1. Alpha Levels Used in Tests of Assumptions

Test	Number of Observations ^a	α When Design Is Balanced	α When Design Is Unbalanced ^b
Normality	N = 2 to 9	0.10	0.25
	N = 10 to 19	0.05	0.10
	N = 20 or more	0.01	0.05
Equality of Variances	n = 2 to 9	0.10	0.25
	n = 10 or more	0.05	0.10

Modified from U.S. EPA 1994

^a N = total number of observations (replicates) in all treatments combined; n = number of observations (replicates) in an individual treatment

^b $n_{\max} \leq 2n_{\min}$

Appendix B.
Raw Data Sheets

Appendix C.
Sample Submission Forms